

MOLECULAR DISSECTION OF FERTILISATION SIGNALLING WITH THE AID OF TYROSINE-KINASE-SPECIFIC INHIBITORS**KEN-ICHI SATO^{1,2}, TETSUSHI IWASAKI¹ and YASUO FUKAMI^{1,2,3}**¹The Research Center for Environmental Genomics, ²Department of Biology, Faculty of Science, ³The Graduate School of Science and Technology, Kobe University, Kobe 657-8501, Japan

Fertilisation is triggered by sperm-egg interaction and fusion that which initiate a transient rise(s) in the free intracellular calcium ($[Ca^{2+}]_i$) that is responsible for a series of biochemical and cell biological events, so called "egg activation". Calcium-dependent egg activation leads to the initiation of the developmental programme that culminates in the birth of individuals. A growing body of knowledge has uncovered the molecular mechanism underlying the sperm-induced transient $[Ca^{2+}]_i$ increase(s) to some extent; namely, in most animals so far studied, a second messenger inositol 1,4,5-trisphosphate (IP_3) seems to play a pivotal role in inducing $[Ca^{2+}]_i$ transient(s) at fertilisation. Despite several years of extensive studies, however, signaling mechanism used by sperm to initiate IP_3 - $[Ca^{2+}]_i$ transient pathway has not been elucidated. To approach this problem, we have employed African clawed frog, *Xenopus laevis*, as a model animal and conducted several experiments designed specifically to determine a role of the Src family protein-tyrosine kinases in the sperm-induced egg activation. *Xenopus* egg fertilisation is accompanied by a rapid and transient rise of tyrosine phosphorylation of egg proteins [1, 2]. Such sperm-induced protein-tyrosine phosphorylation acts upstream of $[Ca^{2+}]_i$ transient, because eggs treated with tyrosine kinase inhibitors can not undergo $[Ca^{2+}]_i$ transient and egg activation in response to fertilization [2, 3]. We have identified a *Xenopus* orthologue of *c-src* proto-oncogene product/protein-tyrosine kinase that is activated transiently after fertilization and that is responsible for the sperm-induced tyrosine phosphorylation of egg proteins [3]. Further experiments have also demonstrated that phospholipase $C\gamma$ is an important component that connects the sperm-induced activation of Src kinase and $[Ca^{2+}]_i$ transient [4, 5], and that egg membrane rafts act as a functional platform for sperm-egg interaction and subsequent Src activation [6]. This lecture will address biochemical identification and molecular cloning of *Xenopus* Src, and the signalling mechanism of the Src-dependent egg activation that has been established with the aid of several tyrosine-kinase-specific inhibitors.

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